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## Integrase-defective Lentiviral Vectors as a Delivery Platform for Targeted Modification of Adenosine Deaminase Locus.

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### Public Summary:

The disease commonly known as 'bubble boy disease' is often caused by a single mutation in the patient's genome. While there have been gene therapies developed for the disease, a true cure has not been reported. In this study, we aimed towards finding a safe and effective way to reverse the disease-causing mutation. We used a "cut-copy-paste" approach to "correct" the mutations. This was achieved by novel proteins called zinc-finger nucleases, which act as molecular scissors to cut the DNA near the mutation. We use the cell's own machinery to repair the cut, but using the correct template, to correct the mutation. This method for so called "genome editing" is becoming more and more popular nowadays. But there are still some areas that need to be sorted out. One such area is about delivering these genome-editing reagents to the cells. We looked at integrase-defective lentiviral vectors, which are non-pathogenic viruses that can deliver cargo to cells for a short time. Using the temporarily-delivered nucleases and donor templates, we can achieve genome editing. This study focused on optimizing the use of these vectors, and showed that we can edit the genome at low efficiency. However, there is room for improvement, especially when it comes to using this approach in stem cells. Our studies contribute to the ever-growing body of research on such genome editing strategies.

### Scientific Abstract:

We investigated the use of integrase-defective lentiviral vectors (IDLVs) for transient delivery of zinc finger nucleases (ZFNs) and donor templates for site-specific modification of the human adenosine deaminase (hADA) gene. Initially, we constructed IDLVs carrying ZFN monomers (Single-IDLVs) and found them to be able to deliver their gene-editing payload to K562 cells successfully upon cotransduction, with minimal cytotoxicity. To simplify delivery, we designed an IDLV construct to deliver both ZFN monomers from the same vector (Double-IDLV). However, this construct in its original state was prone to rearrangements of the vector genome, resulting in greatly reduced functionality; this was due to recombination between highly similar ZFN monomers arranged in tandem. We modified the Double-IDLV constructs to reduce recombination and restored simultaneous delivery of both ZFNs. We also tested an IDLV construct for delivery of donor templates and demonstrated its efficacy for gene modification. In summary, we highlighted the importance of modifying vector design for co-delivery of highly similar sequences inherent to genome-editing nucleases, and demonstrated significant improvement in the use of IDLVs for delivery of ZFNs and donor templates for genome modification. Molecular Therapy (2013); doi:10.1038/mt.2013.106.

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